# Repolarization Matters: Mutations in the Kv4.3 Potassium Channel Cause *SCA19/22*

In this issue of *Annals of Neurology*, Duarri et al[1] and Lee et al[2] report the identification of a gene that when mutated causes a new form of spinocerebellar ataxia (SCA). SCAs comprise a group of neurodegenerative diseases that primarily affect cerebellar neurons, but can also involve other neuronal groups such as the extrapyramidal systems, brainstem, and motor neurons. By convention and in contradistinction to Parkinson disease (*PARK*) and dystonia (*DYT*) genes, the gene symbol *SCA* is reserved for autosomal-dominant forms of ataxia. A great number of *SCA* genes have been identified, with the polyglutamine ataxia genes representing the largest group. Worldwide, *SCA3* and *SCA6* are the most common *SCA* genes, followed by *SCA2* and *SCA1* (reviewed in Whaley et al[3] and Tsuji et al[4]).

In prior studies, both groups had independently mapped a new ataxia locus to partially overlapping segments of human chromosome 1. This explains the confusing designations of *SCA19*[5] and *SCA22*[6] for what in the end turned out to be mutations in the same gene. Using the 2 original large families and several subsequent smaller families, the 2 groups identified mutations in *KCND3*. *KCND3* encodes Kv4.3, a Shal-related voltage-gated potassium channel involved in the transient outward A-type $K^+$ current in neurons and cardiac myocytes.[7]

The 2 groups employed very similar strategies to identify the novel gene, making use of exome capture and next-generation sequencing technology. Whole exome sequencing is now increasingly used for gene identification and was recently successfully utilized to identify a new dominant ataxia in a single Chinese family.[8] Both groups queried variation in expressed parts of the genome including a (near) totality of all protein-coding regions in patients and unaffected individuals. The resulting tens of thousands of variants were filtered by their absence in the ever-growing repositories of normal sequence variants and their location in the candidate region on chromosome 1. When these variants, numbering in the teens after the first filtering step, were further filtered by their likely effect on protein function and cosegregation with the disease in large families, only a single variant per respective family remained.

The mutations identified by whole exome sequencing and by subsequent targeted resequencing in unknown ataxia cases are summarized in the Table. This table also lists commonly used criteria that serve to differentiate benign variants from disease-causing mutations. These encompass genetic criteria (cosegregation of the variant with the disease, recurrence, absence in controls) and functional criteria (evolutionary conservation of the altered amino acid, in vitro effects). The distinction of variants and mutations is neither absolute nor straightforward and relies on a preponderance of evidence. For example, absence of the variant in controls cannot be an absolute criterion; in Parkinson disease there are mutations with nonpenetrance such as *LRRK2* Gly2019Ser that are found in unaffected individuals.[9] Cosegregation with the disease can only be ascertained in large families. Functional analysis is helpful, but in vitro analysis in the relevant cell type is not always possible or can lead to false positives (reviewed in Figueroa et al[10]).

For most of the *KCND3* variants identified by the 2 groups, one can be certain that they represent disease-causing mutations. Only for 2 of the *KCND3* mutations (p.V338E p.T377M) should additional evidence be provided, either by identifying recurrent mutations or by showing functional deficits. Of note, *KCND3* mutations occurred worldwide and in individuals with different ethnic backgrounds. Two of the mutations were recurrent in different ethnic groups. Although a larger sample of ataxia patients will have to be examined, it is likely that *KCND3* mutations will be a relatively rare cause of inherited and sporadic ataxias as compared with ataxias caused by DNA trinucleotide repeat expansions.

This represents the second SCA-causing mutation to be identified in a subunit of a voltage-gated potassium channel. The first was in the gene *KCNC3*, which encodes Kv3.3, an A-type $K^+$ channel that also generates transient outward current.[11] Although it is too early to say with certainty, it seems probable that the mutations identified in *KCND3* in the present studies and those in *KCNC3* may have very similar pathophysiological mechanisms. All voltage-gated potassium channel subtypes are coassembled from 4 similar subunits encoded by families of potassium channel genes (eg, Kv4 family members can coassemble but Kv1 and Kv4 cannot). This quarternary

 

TABLE . *KCND3* Mutations: Genetic and Functional Characteristics

| Mutation | Segregation in >5 Affected Subjects | Recurrent | De Novo Mutation | Absence in Controls | Ethnicity | Conserved Amino Acid | Functional Defects | Reference |
|---|---|---|---|---|---|---|---|---|
| p.T352P | Yes | No | No | Yes | Dutch | Yes | Yes | 1 |
| p.M373I | No | No | No | Yes | Dutch | Yes | Yes | 1 |
| p.S390N | No | No | No | Yes | Dutch | Yes | Yes | 1 |
| p.F227del | Yes | Yes | No | Yes | Chinese; French | Yes | Yes | 2 |
| p.G345V | No | Yes | No | Yes | US AJ; Japanese | Yes | N/A | 2 |
| p.V338E | No | No | No | Yes | Japanese | Yes | N/A | 2 |
| p.T377M | No | No | No | Yes | Japanese | Yes | N/A | 2 |

AJ = Ashkenazi Jewish; N/A = not applicable.

protein structure means that 1 defective subunit can lead to a dominant negative functional effect consistent with the dominant genetic inheritance patterns of *SCA* genes. For *SCA13*, it is known that 2 of the mutations exert a dominant negative effect, rendering the channels much more difficult to open in response to depolarization.[10–12] Cell biological and electrophysiological findings in the Duarri and Lee papers are consistent with a dominant negative mechanism of action, but more electrophysiological experiments will be necessary, using heteromultimeric channels and examining all of the identified mutations, before a dominant loss of function mechanism can be confirmed.

For all but 1 of the *SCA13* and all of the *SCA19/22*-linked mutations, the prediction is that action potentials in affected cerebellar neurons are prolonged, possibly leading to more calcium influx and calcium-dependent toxicity (1 of the *SCA13* mutations seems to facilitate Kv3.3 current).[11] However, despite the striking similarities in the functions of the normal Kv3.3 and Kv4.3 channels and their implication in similar channelopathies, there are some intriguing differences. First, the patterns of expression of these channel genes are slightly different, with Kv4.3 being expressed in the heart and linked to Brugada syndrome, a genetic cardiomyopathy. Of note, the *KCND3* mutations associated with Brugada syndrome have been associated with gain of positive function effects on the channel increasing I(to) current in the heart.[13] Second, Kv3 and Kv4 channels are likely to be subject to different postranslational modifications. Kv4 channels have been reported to interact with KChIP accessory subunits that confer regulation by intracellular calcium provided by particular voltage-gated calcium channel subtypes.[14] Kv4.3 channels also have a very specific subcellular localization pattern in cerebellar inhibitory interneurons, concentrating at sites where climbing fiber contacts are made onto neighboring Purkinje cells.[15] The import of these highly specific functional features of Kv4.3 are not understood, but it is conceivable that they will be relevant for some of the clinical features that may distinguish *SCA13* and *SCA19/22*.

The phenotype and age of onset in the reported *SCA19/22* families are variable. Although most patients show onset in middle age and a predominantly cerebellar syndrome, some patients have onset after age 50 years or have extracerebellar signs and symptoms. Imaging studies are reported as showing mild to moderate cerebellar atrophy without volume loss in the brainstem. Initial observations by both groups suggest the existence of phenotype–genotype correlations. Patients with mutations leading to complete absence of *KCND3* function appear to have a more severe phenotype, with extracerebellar features such as cognitive impairment and myoclonus. Given the relatively small number of patients with a given mutation, however, one cannot discount the influence of genetic background and environmental exposures on at least a portion of the phenotypic variance.

The *KCND3* gene will soon be available for clinical genetic testing. In contrast to testing for CAG-repeat expansions, where clear ranges exist for interpretation (normal–premutation–mutation), the interpretation of changes identified by sequencing of disease genes can be fraught with difficulties. Only rarely can all or most of the criteria in the Table be fulfilled by a newly discovered variant, and the laboratory (correctly) designates the change as a "variant of unknown significance." The distinction between variants of unknown significance and mutations may not be clear to patients. In the experience of 1 of us (S.-M.P.) with *SCA13* testing, patients often tend to equate variant with disease-causing mutation,

which can lead to incorrect diagnoses, false prognostication, and inappropriate genetic testing of other family members. With clinical use of whole exome or whole genome sequencing, we can anticipate a need for education of physicians, genetic counselors and, above all, patients and their family members.

## Potential Conflicts of Interest

S.-M.P.: board membership, AAN; consultancy, speaking fees, Athena Neurosciences; grants/grant pending, NIH, NAF; patents, stem cell patent pending; royalties, Cedars-Sinai Health Systems.

**Stefan-M. Pulst, MD, DrMed**

Department of Neurology
University of Utah
Salt Lake City, UT

**Thomas S. Otis, PhD**

Department of Neurobiology
University of California Los Angeles
Los Angeles, CA

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